Characterization of Functional Genes GS3 and GW2 and their Effect on the Grain Size of Various Landraces of Rice

Haroon Rasheed
Zhejiang University
Sajid Fiaz (sfiaz@uoh.edu.pk)
The University of Haripur https://orcid.org/0000-0001-9097-4359
Muhammad Abid Khan
Zhejiang University
Sultan Mehmood
University of Science and Technology
Faizan Ullah
University of Science and Technology, Bannu
Shahid Ullah Khan
Huazhong Agriculture University: Huazhong Agricultural University
Sumbul Saeed
Huazhong Agriculture University
Tabassam Yaseen
Bacha Khan University Charsadda
Abdul Qayyum
The University of Haripur

Research Article

Keywords: Grain quality and yield, Genetic factors, Mutant alleles, Genotypic characterization

Posted Date: November 10th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-1024333/v1

Abstract

Grain size is an essential factor in grain quality and yield. In the existing agricultural lands in Pakistan and even all over the world, genetics in rice works better for yield potential and quality improvement. GS3 and GW2 with functional mutation responsible for grain size in rice. In the current study, 17 different Pakistani landraces of various genetic and geographic backgrounds were evaluated for grain phenotypic traits (thousand-grain weight, length, width, and thickness) and characterized genotypes for GS3 gene (grain length) and GW2 (grain width). The two accessions JP5 and Bas370, were used as control. Phenotypic data revealed the range for grain weight from 16.86g (Lateefy) to 26.91g (PS2), grain length ranged from 7.27 mm (JP-5) to 12.18 mm (PS2), grain width ranged from 2.01 mm (Lateefy) to 3.51 mm (JP5), and grain thickness ranged from 1.79 mm to 2.19. Pearson correlation revealed a negative and significant correlation between grain width and length. There was no significant correlation between grain length and 1000-grain weight and grain width. LSD test displayed that the means of three variables grain length, grain width, and 1000-grain weight were statistically different from one another except grain width and grain breadth. GS3 is a negative regulator of grain length. Fifteen accessions GA-5015, PS-2, Swat-1, Swat-2, DR-2, Dilrosh, Malhar-346, Kashmir Basmati, Rachna Basmati, KS-282, Basmati-370, KSK-133, KSK-434, MG-Basmati, and Lateefy, carried the domesticated allele of GS3 while JP5 and Fakhr-e-Malakand carried the dominant allele. Similarly, the GW2 is a negative regulator of grain width. Fifteen accessions, i.e., Bas-370, GA-5015, PS-2, Swat-1, Swat-2, DR-2, Dilrosh, Malhar-346, Kashmir Basmati, Rachna Basmati, KS-282, KSK-133, KSK-434, MG-Basmati, and Lateefy carried the dominant allele while JP-5 and Fakhr-e-Malakand carried the mutant allele. The current phenotypic evaluation of the Germplasm revealed a diverse range of grain size of Pakistani landraces and also suggests that the selection of grain length in Pakistani landraces was independent of 1000-grain weight. The accessions with genotypic characterization will aid in marker-assisted breeding programs to break the stagnant yield prevail for the last few decades in Pakistan.

1. Introduction

Rice is used as an essential staple food worldwide [1]. More than 90% of total rice is utilized and consumed with in Asia, where 60% of the world population resides [2]. The class *Oryza* belongs to the tribe *oryzeae* of the family *Poeceae,* which consists of 12 genera and more than 70 species. The genus *Oryza* contains 22 perceived species 20 of total are wild ones while 2, *Oryza sativa,* and *Oryza glaberrima* are cultivated ones. *Oryza sativa* is generally cultivated in Asia, South and North American, Middle Eastern, European Union, and African nations. However, *Oryza glaberimma* is grown exclusively in West African nations [3]. Rice has transformed into a valuable model structure for monocot plants because of having littlest genome of about 400 Mb [4, 5]. Asian cultivated species *Oryza sativa* evolved from its annual wild species *Oryza nivara*, which in turns evolved from a perennial wild ancestor *Oryza rufipogon*. Similarly, *Oryza barthii* and *Oryza longistaminata* are believed as the ancestors of the African domesticated rice, *Oryza glaberrima*. Both the cultivated rice seems to be domesticated in the same parallel evolutionary pathway [6]. In a recent couple of decades, China set up a national super plan on the

advancement of super rice in 1996 and had effectively coordinated through hybrid power. The system was embraced for improved yield with the help of super rice reproducing by a joint coordinated effort between IRRI, Philippines, and China. This plan was begun in 1996-2000 as Phase-I through focused yield of 10.5 tons per ha [7], from 2001-2005 was Phase-II with 12 tons per ha and third it underway in Phase-III in 2011-2015 with 13.5 tons per ha of focused yield [8]. The specialized methodologies utilized in this plan were morphological enhancement between sub specific heterotic restorers and usage of ideal qualities from wild rice by methods for biotechnology. The morphological enhancement comprises of large leaf shade, inferior panicle point, and greater size of panicle [7]. Rice is the second key cash crop in Pakistan, which has an aggressive improvement in the creation of aromatic basmati rice. Punjab and Sindh are the significant rice generation regions, representing 56% and 39% of aggregate creation, individually. The finest long-grain type of rice is Basmati, profoundly esteemed for its smell and taste and solely developed in specific areas of the Punjab. In Pakistan, all things considered, normal rice yield nearly low than China, India, South Korea, USA, Vietnam, North Korea, Bangladesh, Egypt, Brazil, Iran, Philippines, and so on. It is important to understand to increase food safety locally and keep the monopoly in global markets [9]. So, it is essential to boost the yield and maintain the value of rice in Pakistan.

The yield of rice is predominantly represented by 3 most important constituents, i.e., grain weight, grains number per panicle and tillers quantity per plant [10]. The weight of grain is dependent upon grain size (grain length, grain width, and length to width ratio). Grain weight is frequently represented by thousand-grain weight in reproduction applications and by grain length, width and thickness as well [11]. Beyond grain contribution to yield, the appropriate grain size of rice is also preferable among the most consumers and worldwide market.

Rice grain quality comprises a few characters: cooking quality, aroma, grain appearance, milling, and dietary quality etc. Among these, the appearance, eating and cooking characteristics globally create critical commodity issues that adversely affect production. The appearance of rice grain is chiefly indicated by the size of the grain as characterized by grain length (GL), grain width (GW) and the length vs width ratio (LWR), and whiteness of the endosperm [12]. However, the population is increasing day by day. On the other hand, various variables, such as water shortage, soil saltiness, diseases, climate change, and the decreased fertile land zone, will increase inadequate food in the upcoming 50 years [12]. That is why grain size is also an essential agronomic attribute for artificial selection. Breeders will be likely to select plants with large seed estimates in rice breeding in light of the fact that grain size firmly identified with the yield and superiority of rice [13]. However, it is hard for breeders to enhance grain size effectively by phenotypes, since the characters of grain quality are quantitative [14, 15]. Within existing agricultural lands in Pakistan and around the world, genetics in rice works better for yield potential and quality maintenance and might be the perfect way to enhance yield.

Based on the above assumptions, the following objectives of the study were, (1) To phenotype the selected genotypes for grain size. (2) To screen functional mutation in *GW2* and *GS3* responsible for grain size in rice.

2. Results

2.1. Morphological results

Morphological evaluation of selected Germplasm revealed the highest 100-grain weight of (26.74 g) for PS2, while the lowest (20.06 g) were recorded for Rachna Basmati. Similarly, the highest grain length (12.18 mm) was recorded for PS2. Although JP-5 showed the shortest grain of (7.27 mm) but had the widest grain (3.51 mm) among the Germplasm. Narrowest grains of (2.21 mm) were recorded for Rachna Basmati. We also measured the grain thickness, and the JP-5 (2.19 mm) showed the highest value, while Rachna Basmati showed the lowest (1.90 mm) measure for grain thickness. Grand mean of 22.87g, 9.59mm, 2.47mm, and 1.94mm were recorded for grain weight, respectively. The highest standard deviation was recorded for grain weight while the lowest was recorded for grain thickness. However, the highest coefficient of variation was recorded for grain width, while the lowest was recorded for grain thickness (Table 1).

Table 1

Grain evaluation for Grain weight, grain length, grain width, and grain thickness

S.No	Landraces	Grain weight (g)	Grain Length (mm)	Grain Width (mm)	Grain Thickness (mm)
1	JP-5	24.78	7.27	3.51	2.19
2	Fakhr-e-malakand	25.69	8.06	3.10	2.01
3	GA-5015	25.44	11.15	2.30	1.91
4	PS-2	26.74	12.18	2.44	1.85
5	Swat-1	18.35	7.99	2.81	1.93
6	Swat-2	25.42	9.83	2.45	1.99
7	DR-2	24.67	9.45	2.49	1.88
8	Dilrosh	24.91	10.55	2.45	1.96
9	Malhar-346	19.75	8.80	2.22	1.9
10	Kashmir Basmati	20.13	9.023	2.21	1.92
11	Rachna Basmati	20.06	9.11	2.21	1.90
12	KS-282	26.00	10.43	2.44	2.02
13	Basmati-370	19.02	9.33	2.17	1.84
14	KSK-133	26.91	9.82	2.64	2.09
15	KSK-434	23.70	10.09	2.37	1.98
16	MG-Basmati	20.38	10.78	2.14	1.79
17	Lateefy	16.86	9.13	2.01	1.9
	Grand mean with standard deviation	22.87+3.32	9.59+1.23	2.47+0.37	1.94+0.09
	CV	14.52	12.85	15.22	5.02
	MAX	26.91	12.18	3.51	2.19
	MIN	16.86	7.27	2.01	1.79

Information based on an association of various traits is essential to gather them for yield and quality improvement in rice. To understand the nature and magnitude of the association between the four-grain traits, i.e., 1000-grain weight, grain length, grain width, and grain thickness were calculated in Table 2.

Pearson correlation revealed the highest positive association between grain width and grain thickness, and after that thousand-grain weight and grain thickness, both correlations were highly significant and significant, respectively. However, the highest negative correlation was observed between grain length and width, which were statistically significant, followed by between grain length and grain thickness, which

were not statistically significant. The correlation between thousand-grain weight and grain length were not statistically significant. Further, the correlation revealed that the contribution of grain traits in the selected Germplasm to 1000-grain weight one of the main constituents of yield, were in order TGW=GT>GW>GL (Table 2).

Table 2

Pearson correlation among the grain phenotype

	TGW	GL	GW
GL	0.3818		
	P=0.13		
GW	0.4545	-0.532	
	P=0.06	P=0.02	
GT	0.4908	-0.4581	0.7748
	P=0.04	P=0.06	P=0.00

TGW= Thousand Grain Weight, GL= Grain Length, GW= Grain Width, GT= Grain thickness. LSD test revealed that the three traits mean compared were statistically different from one another except grain width and grain thickness at P=0.05 (Table 3).

Table 3

LSD test for the grain phenotypes

	TGW	GL	GW
GL	13.28*		
GW	20.40*	7.12*	
GT	20.92*	7.64*	0.52
TGW= Thousand Grain Weight, GL= Grain Length, GW= Grain Width, GT= Grain thickness			

The LSD test which basically uses a t-test to compare the means revealed that the traits 1000-grain weight, grain length, and width were statistically different from one another. Pearson correlation showed a negative correlation of grain length with grain width, and from the picture of accessions (Figure 1), it is evident that profoundly meaningful Germplasm was selected to distinguish the individual effect of *GS3* and *GW2* on their respective traits.

2.2. Molecular genotyping

2.2.1. GS3 C-A allele genotyping

The extracted DNA was amplified for SF28 respective for *GS3* amplified ~140bp fragment size. After the amplification, the product size was digested with a *PST1* restriction enzyme. Those accessions which carry wild type allele C nucleotide carries a splicing site CTG**C**AG for *PstI* which cleaved the wild type (JP-5 as positive control) PCR product into(~110bp) and (~30bp) fragment size while did not digest the domesticated allele carry C-A substitute i.e., CTG**A**AG (Figure 2).

The accessions with a non-digested fragment of (~140bp) (Bas-370 as negative control) fragments were represented with (+) for presence and those with digested fragments of (~110bp) and (~30bp) were represented with (-) for absence. Out of 17 accessions, Germplasm the two accessions JP-5 and Fakhr-e-Malakand carried dominant allele for while the remaining fifteen accessions i.e., GA-5015, PS-2, PS-2, Swat-1, Swat-2, DR-2, Dilrosh, Malhar-346, Kashmir Basmati, Rachna Basmati, KS-282, Basmati-370, KSK-133, KSK-434, MG-Basmati, and Lateef carried the domesticated allele (Table 4).

Table 4

List of accessions carrying *GS3*

S.No	Landraces	*GS3*	S.No	Landraces	*GS3*
1	JP-5	+	10	Kashmir Basmati	-
2	Fakhr-e-malakand	+	11	Rachna Basmati	-
3	GA-5015	-	12	KS-282	-
4	PS-2	-	13	Basmati-370	-
5	Swat-1	-	14	KSK-133	-
6	Swat-2	-	15	KSK-434	-
7	DR-2	-	16	MG-Basmati	-
8	Dilrosh	-	17	Lateefy	-
9	Malhar-346	-			
(+) for the presence of dominant *GS3* gene (-) for the absence of *GS3*					

3.2.2. GW2 allele genotyping

The *GW2* marker, when amplified, also revealed a monomeric fragment size of 51bp for each accession. The amplified product digest with APO1 exhibited two different banding patterns, i.e., one a 51bp, which did not carry the restriction site for *APO1,* and the other cleaved of 30bp and 21bp [18] (Figure 3).

The two accessions JP5 and Fakhr-e-Malakand carries the domesticated allele while Bas-370, GA-5015, PS-2, PS-2, Swat-1, Swat-2, DR-2, Dilrosh, Malhar-346, Kashmir Basmati, Rachna Basmati, KS-282, KSK-133, KSK-434, MG-Basmati, and Lateefy carried the dominant allele for *GW2* (Table 5).

Table 5

List of accessions carrying *GW2*

S.No	Landraces	*GW2*	S.No	Landraces	*GW2*
1	JP-5	-	10	Kashmir Basmati	+
2	Fakhr-e-malakand	-	11	Rachna Basmati	+
3	GA-5015	+	12	KS-282	+
4	PS-2	+	13	Basmati-370	+
5	Swat-1	+	14	KSK-133	+
6	Swat-2	+	15	KSK-434	+
7	DR-2	+	16	MG-Basmati	+
8	Dilrosh	+	17	Lateefy	+
9	Malhar-346	+			
(+) for the presence of dominant *GW2* gene (-) for the absence of *GW2*					

3. Discussion

The grain size of *Oryza rufipogun* and *O. longistiminata,* which are the wild ancestors of domesticated rice has small and round grain because natural selection often favors such phenotypes to possess a high level of fertility and dispersal for their survival [19]. Thus, grain size has a vital role in the domestication of cereal crops [20, 21]because grain size, besides its role in grain quality, independently regulates grain yield by increasing grain weight [22, 23]. Domestication has also greatly enhanced the variability of grain size and shape due to the human need for rice as a staple food in diverse natural and cultural environments [20]. Therefore, manipulating grain size, not only in rice but in many cereal crops, is an effective strategy for increasing yields and quality. In the current study divergent range of grain size in Pakistani landraces was characterized.

The present accessions showed a negative but strong correlation between grain length and width. Similarly, [24] also reported a highly significant and negative correlation between grain length and width in 113 various Pakistani Germplasm of rice. Similarly, in Indian Germplasm, [25] also reported a negative but weak correlation between these two traits. On the other hand, the correlation studies of [25] also revealed a highly significant correlation between grain length and 1000-grain weight.

Similarly, [24] reported no correlation between grain length and thousand-grain weight. Our results concur with the latter study. In Pakistan, mostly long-grain varieties are grown where people usually prefer long grain. The shreds of evidence suggested the phenomena, preference, and devotion of the plateau ancient conventional farmer to grain quality that selected long-grain rice independent of grain weight. Further, [24] also reported that 1000-grain weight is strongly dependent upon grain width. [25] reported no significant correlation between these two traits. However, our results revealed that these two traits were non-significant. These some contradictions in relationship between the traits of grain size might be due to the variation in the selection of Germplasm in studies.

Artificial selection has a domesticated grain length in rice [20]. To understand the genes regulating long-grain, along with its influence on phenotype is indeed important to increase grain size in rice while incorporating in any breeding programs. *GS3* has been earlier reported in diverse genetic backgrounds and environments [20, 23, 26]. In the present study, we reported a high frequency of CTG**A**AG allele than CTG**C**AG allele for *GS3* in long-grain rice. Many studies also reported the widespread *GS3* in the global collection of rice [20, 27]. The result indicates that C-A mutation has an ancient origin that played a vital role in diversity and cultivated rice domestication. [28] transferred the cDNA of *GS3* in Minghui63 making transgenic lines. Their homologues transgenic lines showed significant reduction in the grain size. [25] also found 37.07% frequency of C-A mutation in 89 Indian origins Germplasm. In the present study we even found the domesticated allele for a Swat-I, and Malhar-346 which have grain length of 7.99mm, and 8.80mm, respectively. Previous studies also reported that many genes are responsible for the regulation of grain length. Additionally, [29] also reported gene to gene interaction that *GS3* C-A allele makes efficient another grain size regulator gene *GS7,* and the latter gene is ineffective in the presence of C allele. Besides *GS3* importance for the presence of in current Germplasm, grain length is a quantitative trait.

GW2 is a major gene responsible for grain width and enhances grain yield, grain filling, and grain weight in rice [30-33]. *GW2* is a negative regulator of grain size. The present primer for *GW2* showed significant results for grain width in the current study. [33] recently used another STS marker (WOO4) for *GW2* allele genotyping in 89 accessions and reported two alleles with a frequency of 73.03% and 29.94% respectively. However, they did not find any significant allelic variation for the grain width and recommended that the WOO4 marker showed not be used further in any breeding program. [18] used the same primer as in the study reported meaningful results. [18] further testified the *GW2* protein expression, which is dominant in Kasalath (2.48 mm), which negatively regulates grain width and *TD70* carries the mutant allele showing grain size of (4.42 mm).

4. Materials And Methods

The current study was conducted at Department of Botany, University of Science and Technology Bannu in 2018. For this purpose, 17 different landraces, i.e., JP-5, Bas-370, GA-5015, PS-2, Swat-1, Swat-2, DR-2, Fakhr-e-Malakand, Dilrosh, Malhar-346, Kashmir Basmati, Rachna Basmati, KS-282, KSK-133, KSK-434, MG-Basmati, and Lateefy were chosen among Pakistani rice Germplasm that showed highly significant

variation for grain size, including grain length and breadth. The two accessions JP5 and Bas370, were used as control. To get fresh seeds, the seeds of each sample were grown in greenhouses under a controlled environment. Twenty days old seedlings were transplanted to pots each sample in three replications. Mature paddy seeds were collected from each accession.

4.1. Morphological characterization

4.1.1. Measurements of grain traits

Paddy rice was air-dried and stored at room temperature for about 3 months before testing. Completely filled grains were used for measuring grain length, width, thickness, and weight. 10 randomly selected grains from each plant were aligned length-wise along a Vernier caliper to measure grain length. The distance was calculated from the base to the tip of the grain. The distance was calculated to measure grain width over the fertile lemma and palea at the widest point with Vernier caliper for an average of 10 representative grains for each accession. The thickness of grain was also calculated for each grain of 10 representative seeds separately via Vernier caliper, and their values were averaged and used as the measurements for the plant. Grain weight was calculated based on 200 grains and converted to 1,000-grain weight for simplicity of comparison with other studies [16].

4.2. Statistical analysis

Statistical analysis was carried out using statistical package Statistix 8.1 for Pearson correlation. Later the data was subjected for Analysis of variance and LSD test to report whether the means of two variables were statistically significant or not.

4.3. Molecular characterization

DNA extraction was performed from young leaf tissue using a modified CTAB method [17]. To validate the expected DNA fragment Gel electrophoresis was performed. The two primers *GS3* and *GW2* (Supplementary table) were used to detect the existence of corresponding grain length and width gene on chromosome 3 and 2, respectively. We prepared a master mix for seventeen samples of 18 µl for each sample. The individual sample reaction contains, 12.24 µl of water, 2mM Mgcl2, Taq buffer of 4 µl, 0.2 µl of each forward and reverse primer of 20mM, 0.4 µl of dNTPs, and 0.16 µl of Taq polymerase. For PCR of each sample, 2 µl of DNA from each accession was added to each 18 µl reaction sample. Each sample with accession DNA was subjected in small tubes to thermocycler PCR.

PCR condition was set for 40 cycles with Pre-melting of 94ºC for 2 min, cycle melting for 30 seconds at 94ºC, annealing temperature for 30 sec on 55ºC and at 72ºC for 30 seconds for template extension. At the end, 5 minutes were fixed for a final extension of remaining templates. The 2µl of amplicons of each

SF28 and *GW2* were run on 1% gel electrophoresis by staining with ethidium bromide. The remaining amplified product of SF28 was digested with restriction enzyme *PstI* while the *GW2* fragment was digested with an *APO1* restriction enzyme. To make the final volume of 15µl, the amplified products of 10µl*GS3* and *GW2* were cleaved with 1 unit of each restriction enzyme with 1X restriction buffer and deionized water. To digest the amplicons completely the mixture was kept at 37 ºC for 4-16 hrs. Polymorphic banding patterns of each amplicon were separated and examined with gel electrophoresis of 6% agarose gel and visualized under gel documentation system. Both the primers were scored (+) for the presence and (-) for the absence of respective genes.

5. Conclusions

The current Germplasm showed a high range for grain length from 7.27 to 12.18 mm and for grain width from 2.21-3.51 mm. The phenotypic correlation further revealed that the grain length in the Pakistani Germplasm independently evolved of grain weight. PS2, GA-5015, and MG-Basmati are narrow and long grain genotypes while JP-5 and Fakhr-e-Malakand are shortest grains varieties. *GS3* and *GW2* showed significant frequency for grain length, and grain width alleles, and such accessions could be used as marker-assisted selection in a breeding program for both rice yield and quality improvement. However, in addition to *GS3* and *GW2* in some accessions, there might be further genes either fluctuating through the additive effect or interact with one another and the environment.

Declarations

Author Contributions:

Conceptualization, H.R.; M.A.K.; S.M.; F.U.; methodology, H.R.; M.A.K.; S.M.; F.U.; software, H.R.; M.A.K.; S.M.; F.U.; formal analysis, H.R.; M.A.K.; S.M.; F.U.; investigation, H.R.; M.A.K.; S.M.; F.U.; S.F and A.Q provided technical expertise, writing—original draft preparation, S.U.; S.F.; T.Y.; S.M.K.; S.D.; writing review and editing, S.F.; S.D.; R.D.; M.S.; A.E.S.; M.M.H.; M.B.; funding acquisition, M.S.; A.E.S.; M.M.H.; M.B.

Funding:

No funding is involved to conduct this research.

Compliance with Ethical Standards:

This article does not contain any studies with human/animals performed by any of the authors.

Institutional Review Board Statement:

Not applicable

Informed Consent Statement:

Not applicable.

Data Availability Statement:

Not applicable.

Acknowledgments:

The authors extend their appreciation to Taif University for funding current work by Taif University Researchers Supporting Project number (TURSP-2020/119), Taif University, Taif, Saudi Arabia.

Conflicts of Interest:

The authors declare no conflict of interest.

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Supplemental

Supplementary table is not available with this version

Figures

Figure 1

Accessions used in study, 1=PS2, 2= GA-5015, 3=MG-Basmati, 4= Dilrosh, 5= KS282, 6=KSK-434, 7=Swat-II, 8=KSK-133, 9= DR-2, 10= Basmati-370, 11=Lateefy, 12= Rachna Basmati, 13=Kasmir Basmati, 14= Malhar-346, 15= Fakhr-e-Malakand, 16= Swat-I, 17= JP-5

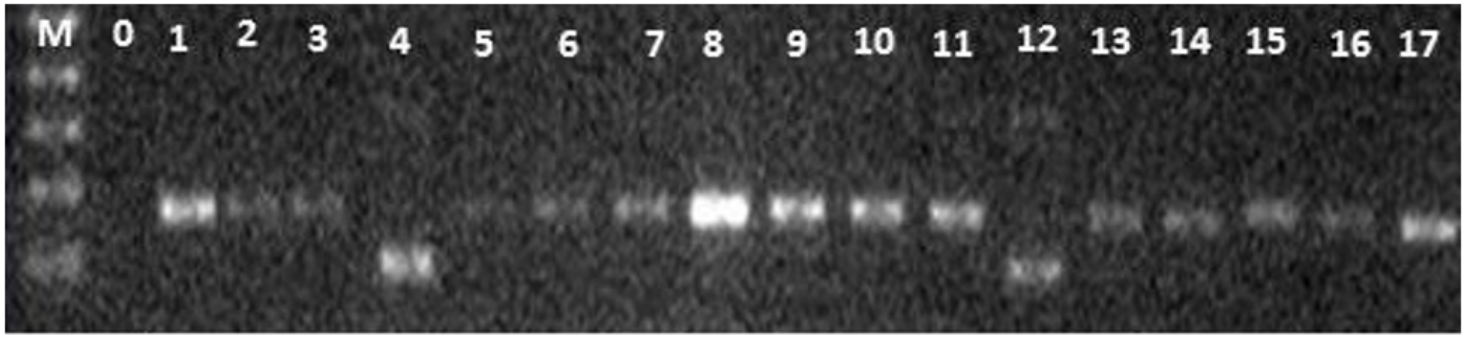

Figure 2

Picture for gene GS3 1=PS2, 2= GA-5015, 3=MG-Basmati, 4= Fakhr-e-Malakand, 5= KS282, 6=KSK-434, 7=Swat-II, 8=KSK-133, 9= DR-2, 10= Basmati-370, 11=Lateefy, 12= JP-5, 13=Kasmir Basmati, 14= Malhar-346, 15= Dilrosh, 16= Swat-I, 17= Rachna Basmati

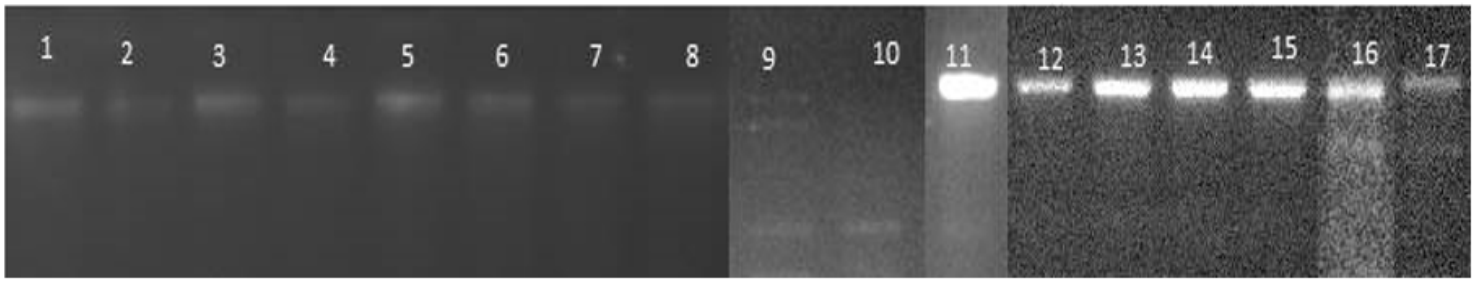

Figure 3

Picture for gene GW2 1=PS2, 2= GA-5015, 3=MG-Basmati, 4= Dilrosh, 5= KS282, 6=KSK-434, 7=Swat-II, 8=KSK-133, 9= JP-5, 10= Fakhr-e-Malakand, 11=Lateefy, 12= Rachna Basmati, 13=Kasmir Basmati, 14= Malhar-346, 15= Basmati-370, 16= Swat-I, 17= DR-2